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Effect of endotoxin on iron absorption

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CORTELL, STANLEY, AND MARCEL E. CONRAD. *Effect of endotoxin on iron absorption.* Am. J. Physiol. 213(1): 43–47. 1967.—Endotoxin caused marked abnormalities in iron absorption and metabolism within hours after the administration of a parenteral dose. The changes observed during the 1st day following injection were unique; there was decreased absorption of iron with a normal intestinal iron content, an accelerated rate of clearance of iron from plasma, and a decreased serum iron concentration. That a generalized cytotoxic effect upon the gut was not the cause of these changes was suggested by the normal intestinal histology and lifespan of mucosal cells, normal absorption of glucose and unchanged excessive absorption of iron by iron-depleted, endotoxin-treated animals. Two days after the administration of endotoxin most abnormalities became normal except that the intestinal iron content increased, and a significant decrease in iron absorption persisted. It was only during this later period that iron-depleted rats had decreased absorption of iron from the gut. We postulated that the acute absorptive defect was caused by a decreased capability to transfer iron from the mucosal cell into the body, whereas the late defect was associated with impaired entry of intraluminal iron into the intestinal absorptive cells.

regulation absorption; radioactive iron; mucosal block; rodents; disseminated intravascular coagulation

THE INJECTION OF ANIMALS with sufficient quantities of endotoxin causes shock and death. Smaller doses produce numerous physiologic changes in both man and animals (32, 38–40). Certain of these changes are beneficial with important biologic effects; such as the induction of fever, stimulation of resistance to infection, and protection against radiation injury. Despite the significance of these reactions, little is known about the mechanisms causing these effects.

Previous investigation in rodents showed that small doses of endotoxin (*E. coli* and *B. abortus*) rapidly deplete the plasma of iron; the serum iron concentration is maximally depressed 6–12 hr after the injection of endotoxin and returns to normal levels 24–36 hr later (1, 23–26). Similar decrements in the serum iron concentration occur with an experimental turpentine abscess, after the injection of typhoid vaccine or during certain acute and chronic infections (7). It has been postulated that these changes occur because of a decreased capability to make iron from destroyed red blood cells available for the synthesis of new hemoglobin (17, 23). That endotoxin reduces phagocytosis by the reticuloendothelial system in rabbits with a similar temporal sequence to changes in the serum iron concentration provides additional evidence for this hypothesis (6).

This study was undertaken to investigate the effect of endotoxin on iron kinetics and the absorption of iron from the gut.

METHODS

Male albino rats, Walter Reed Carworth Farm strain, weighing 200–250 g were used in this study. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed. The lipopolysaccharide from *Escherichia coli* 055:B5, lot no. 150403, was obtained from Difco Laboratories, Detroit, Michigan. This endotoxin was administered intraperitoneally, as an aqueous solution, to rats in a dose of 0.1 mg. Most rats were fed a commercial rat and mouse diet containing about 15 mg iron/100 g of dry wt. Iron deficiency was induced by repeated blood letting and feeding the animals a milk-powder, iron-poor diet (2 mg/100 g). Iron loading was produced 3 weeks prior to study by two intramuscular injections of 25 mg iron as an iron-dextran complex (Imferon).

Absorption studies were performed using a test dose of 0.5 μc ferrous citrate-^{59}Fe with 0.25 mg carrier iron (as ferrous sulfate) in 0.5 ml distilled water. The test dose was injected into the stomach of rats that were fasted overnight through an olive-tipped 17-gauge endoesophageal needle. Whole-body radioactivity (0.8 Mev-∞) was measured in a small whole-body liquid-scintillation detector (Packard ARMAC) 3 hr and 7 days after dosing to determine the percent test dose absorbed by the rats (12). The reliability of this technique was reported (15). Statistical analyses were performed by the *t* test.

In experiments testing the effects of heparin, iron absorption studies were performed in 32 fasted rats. One-half the animals received 100 units of heparin at 6-hr intervals for 18 hr before and 6 hr after admin-

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TABLE 1. *Effect of endotoxin on aspects of iron metabolism at intervals after parenteral dose of 0.1 mg*

	Control	Hours After Endotoxin Administration						
		1	6	12	24	48	72	96
Oral absorption, %	17.8±1.1 (52)	10.0±1.2 (22)	4.5±0.5 (14)	4.5±0.6 (24)	7.9±0.6 (37)	9.6±0.6 (38)	14.2±1.0 (37)	14.7±1.7 (23)
Serum iron, μg/100 ml	185±11 (29)	186±14 (14)	67±8 (16)	47±5 (17)	86±9 (27)	186±8 (10)	170±11 (5)	195±31 (5)
Total iron binding capacity, μg/100 ml	421±15 (29)	462±29 (14)	366±12 (16)	411±17 (17)	437±12 (27)	338±14 (10)	405±15 (5)	437±28 (5)
Plasma iron 59 clearance, T/2 min	62±2.4 (10)		47±2.6 (10)	35±1.6 (10)	49±1.9 (10)	56±1.7 (10)	60±3.2 (10)	
Calculated plasma iron turnover, μg/day	208±2.8		99±3.4	94±2.8	122±3.1	221±4.1	198±5.4	
Gut iron, μg/g	13.0±0.6 (38)	13.2±0.8 (17)		13.2±0.5 (35)	18.8±0.7 (19)	25.0±1.5 (9)	16.9±0.9 (10)	16.9±0.9 (10)

Variation is expressed as the standard error of the mean. Number of animals indicated in parentheses.

istration of the test dose of iron. Eight heparinized and eight unheparinized rats received an intraperitoneal injection of 0.1 mg endotoxin 12 hr before the test dose of iron.

Blood for serum iron measurements and determinations of the total iron-binding capacity was obtained from the retroorbital venous plexus of rats under light ether anesthesia with heparinized capillary tubes. The serum iron and unsaturated iron-binding capacity were determined by the "one-tube method" (37). The total iron-binding capacity was calculated from the serum iron and unsaturated iron-binding capacity.

Segments of gut used for iron analyses were excised from freshly killed animals after an overnight fast. The segments were opened lengthwise and thoroughly rinsed in several changes of iron-free distilled water. The gut segments were prepared for iron analysis in a Virtis homogenizer. The nonheme iron content of intestinal specimens was measured by a modification of the method of Brückmann and Zondek (5). Prior to the development of the color reaction, turbidity was cleared from the chemical reaction mixtures by filtration through 0.45 μ bacterial filters (Gelman Metricel) and a sample blank was utilized (16).

Plasma clearance studies were performed following the injection of 0.2 ml iron 59-labeled rat serum into the dorsal vein of the penis. Labeled serum was prepared by incubating ferrous citrate-^{59}Fe with pooled serum from normal rats. Approximately 1 μc radioiron (0.6 μg of iron per μc) was injected into each animal. The iron-binding capacity of the incubated serum was not exceeded (35). At 10, 20, 30, and 40 min following the injection, the radioactivity in 0.02 ml whole blood obtained from a tail vein was determined in the well-type, crystal-scintillation detector. (Packard Autogamma spectrometer, model 410A). The plasma volume was estimated from the hematocrit and calculated whole blood volume. The fraction of iron removed per hour and the plasma iron turnover per day were calculated from the serum iron concentration, plasma volume, and plasma iron clearance studies (3, 18, 31).

Iron balance studies were performed on rats housed in individual plastic metabolic cages. Dietary consumption of milk containing 0.25 μg iron/ml was measured daily. Four-day fecal and urinary collections were accumulated to determine their total iron content. The excreta were digested by a modification of the method of Hill (21), and the iron content of the digests was measured by a modification of the method of Ramsay (30).

Segments of small intestine were prepared for radioautography and histologic examination as described previously (10, 12, 22). Oral glucose tolerance tests were performed after the intragastric administration of 1 g glucose to animals fasted overnight. The serum glucose concentration was determined by the method of Nelson (29).

TABLE 2. *Effect of 0.1 mg endotoxin on oral absorption of iron, and iron content of duodenum in various states of iron repletion*

	Iron Deficient		Iron Loaded	
	Absorption, % oral dose	Intestinal iron, μg/g	Absorption, % oral dose	Intestinal iron, μg/g
No endotoxin	48.2±5.1	7.9±0.8	4.2±0.6	25.1±2.1
Hours after endotoxin				
12	46.9±3.7	8.0±0.3	3.9±0.4	23.6±1.7
24	32.6±1.8	10.1±0.9	3.7±0.4	26.3±1.9
48	30.5±0.9	10.4±1.0	3.8±0.5	26.5±2.0

Variation is expressed as the standard error of the mean of 10 animals.

TABLE 3. *Effect of heparin on iron absorption by normal and endotoxin treated animals*

	No Endotoxin		Endotoxin 12 Hr Previously	
	No heparin	Heparin	No heparin	Heparin
Percentage of iron[59] absorbed	5.96	5.06	2.30	2.30
	6.17	6.94	2.85	2.31
	9.99	7.07	2.94	2.66
	10.41	8.37	3.64	2.96
	10.54	12.88	3.83	2.99
	10.60	14.04	4.14	3.24
	12.20	14.21	4.43	4.19
	14.70	15.20	6.34	4.40
Mean	10.07	10.47	3.81	3.13
SD	2.89	4.01	1.19	0.79
SE	1.02	1.42	0.42	0.28

TABLE 4. *Effect of 0.1 mg endotoxin administration on oral glucose absorption, 12 and 24 hr after parenteral dose*

	Serum Glucose, mg/100 ml	
	Fasting	½ hr
Control	72±10	122±17
Hours after endotoxin		
12 hr	62±11	138±14
24 hr	86±12	138±6

Variation is expressed as 1 SD.

RESULTS

Iron absorption was measured in normal animals and in rats injected with endotoxin at various intervals before administration of the oral dose of radioiron. The absorption of iron was significantly decreased 1 hr after the injection of endotoxin (10 vs. 17%, $P < 0.01$), and was maximally reduced 6–12 hours after endotoxin (4.5%). Subsequently, more iron was absorbed from test doses of iron, but absorption remained significantly decreased ($P < 0.05$) until 96 hr after endotoxin administration (Table 1). In iron-deficient and iron-loaded rats, the absorption of iron from the gut was unchanged 12 hr after the administration of endotoxin (Table 2). Iron-deficient animals had a significant reduction in the absorption of iron at 24 hr (33 vs. 48%) and 48 hr (31%) after the injection of endotoxin ($P < 0.01$).

The serum iron concentration remained unchanged from normal 1 hr after the administration of endotoxin (186 vs. 185 μg/100 ml). Two hours postinjection, the serum iron concentration was significantly decreased (135 μg/100 ml), and was maximally depressed at 12 hr (47 μg/100 ml). It remained significantly decreased 24 hr (86 μg/100 ml) after the injection of endotoxin. Forty-eight hours after the administration of endotoxin the serum iron concentration was normal (186 μg/100 ml), and remained normal in specimens obtained at later intervals. The total iron-binding capacity of serum was decreased 6 and 48 hr after the injection of endotoxin ($P < 0.05$) (Table 1). This significant reduction in the total iron-binding capacity of endotoxin-treated animals was previously reported by Kampschmidt, Upchurch, and Johnson (26), but was more persistent in their animals than ours.

Chemical measurements of the nonheme iron content of one-quarter of the small intestine showed normal animals had 13 μg iron/g tissue. The intestinal iron content remained normal for at least 12 hr after the administration of endotoxin (Table 1). At 24 hr the iron content of intestinal segments increased to 18.8 μg/g and the maximal concentration was observed at 48 hr (25 μg/g). Thereafter, the iron content decreased, but the values seemed to remain slightly greater than normal 72 and 96 hr after the injection of endotoxin (16.9 μg/g).

Plasma iron clearance studies were performed in fasted, normal animals, and in rats at intervals after an injection of endotoxin. In normal animals, one-half the iron 59 was cleared from the plasma (T/2) in 62 min, and the calculated plasma iron turnover was 208 μg daily. Following the administration of endotoxin, radioiron was cleared from the plasma at an accelerated rate (T/2). However, there was no increase in the calculated plasma iron turnover because of the simultaneous decrease in the plasma iron concentration. Abnormalities were most marked 12 hr after the administration of endotoxin, the plasma iron clearance (T/2) was 35 min and the daily plasma iron turnover was computed to be 94 μg. Subsequently, these measurements changed toward normal values (Table 1).

Body loss of iron was measured by chemical and radioisotopic balance studies of cumulative fecal and urinary collections from rats fed an iron-poor milk diet. Chemical analyses did not demonstrate a significant difference in the excretion of iron between normal and endotoxin-treated animals (45 to 50 μg/day). Likewise, the daily body loss of intravenously injected iron 59 was not significantly affected by the administration of endotoxin (0.2% per day).

Histologic studies of the small intestine and measurements of mucosal lifespan were performed to ascertain if endotoxin caused changes which affected iron absorption. Tritium-labeled thymidine was infused intravenously into normal animals and rats that received a concurrent dose of endotoxin. The duodenum and jejunum were excised from these animals 22 and 32 hr later. Radioautographs were prepared from sections of these guts and showed a similar turnover rate of intestinal mucosal cells in normal and endotoxin-treated animals. Sections of duodenum and jejenum, stained with hematoxylin and eosin, periodic-acid Schiff, and Sudan, showed normal villous architecture in normal and endotoxin-treated animals.

The injection of endotoxin into animals is reported to produce a hypercoagulable state with disseminated intravascular coagulation (28). That this hemostatic

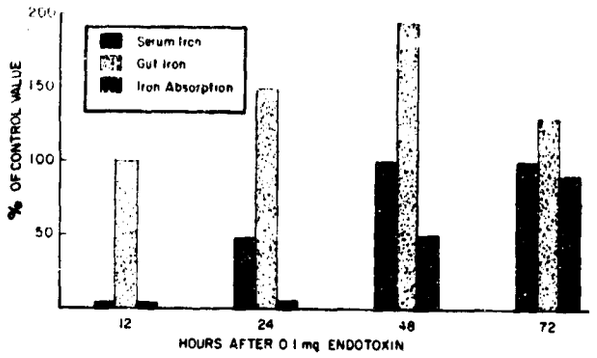

FIG. 1. Measurements of the serum iron concentration, iron content of the duodenum, and iron absorption at intervals after injection of endotoxin are depicted as the percent of values observed in normal untreated rats. Serum iron concentration was markedly diminished at 12 hr and became normal 48 hr after an injection of endotoxin. Iron concentration of the gut was not increased until 24 hr after endotoxin. Thus, serum iron concentration at 48 hr and the total iron content of the duodenum 12 hr after an injection of endotoxin did not seem to be regulators of iron absorption.

defect did not act as an intermediary mechanism for endotoxin to decrease iron absorption was suggested by the failure of heparin anticoagulation to affect either the normal absorption of iron or the decreased absorption caused by the injection of endotoxin (Table 3).

Absorption of glucose was measured in normal and endotoxin-treated rats to ascertain if endotoxin caused generalized malabsorption. The blood sugar was measured in rats that were fasted overnight and ½ hr after the oral administration of 1 g glucose. The absorption of glucose was not impaired in the endotoxin-treated animals (Table 4).

DISCUSSION

The iron content of the body is small, amounting to about 60 ppm (36). This concentration is maintained throughout life by a regulated equilibrium between iron absorption and excretion; consistent alterations in this balance would cause either iron deficiency or siderosis. Although excretion of iron is selective, it is limited; and iron balance is controlled primarily by regulating absorption for body requirements (8, 12, 14, 27).

Iron enters the body primarily through duodenal absorptive cells. In the normal state of iron repletion a controlled amount of dietary iron enters the absorptive cell, a portion is transferred into the body, and the remainder stays within the mucosal cell. In iron deficiency, increased amounts of dietary iron are absorbed and little iron is held by intestinal cells. In iron overload, the absorptive cells accept little iron from the lumen of the gut. Thus, there is regulation of both the quantity of iron that enters the intestinal cell from the lumen of the gut and the amount of iron transferred from the absorptive cell into the body (10-12, 33).

Many factors have been considered important as regulators of iron absorption. That the plasma iron concentration, the total iron-binding capacity, or the hemoglobin concentration of blood are the primary regulators of iron absorption seems unlikely (2, 20); bled humans absorb excessive amounts of iron after laboratory values return to prephlebotomy levels (9). The capability to increase absorption in iron-loaded animals indicates that body stores do not control absorption directly (12). One hypothesis postulates that the iron content of intestinal absorptive cells is important in the regulation of iron absorption (19, 20); an increased body requirement for iron, such as accelerated erythropoiesis, would deplete the intestinal cells of iron and permit increased amounts of dietary iron to enter the cell (11, 34). The transfer of iron from intestinal cells into the body would depend on current iron requirements and might be mediated by the plasma iron turnover (33).

Most factors that change iron absorption require several days before their effects become manifest (4). Contrariwise, endotoxin causes significant changes in iron absorption within 1 hr after injection. That this is not a generalized cytotoxic effect on the intestinal mucosa or vasculature is suggested by the normal histologic appearance of the gut, the normal lifespan of mucosal cells, the normal absorption of glucose, and the capability of iron-deficient, endotoxin-treated rats to absorb excessive amounts of iron.

Arbitrarily, our data can be divided into an acute phase to include the 24-hr period following the injection of endotoxin, and a late phase. During the 1st day, endotoxin-treated normal animals show decreased iron absorption followed by a marked depletion of iron from the plasma. The plasma clearance (T/2) of transferrin-bound iron is rapid, but iron turnover is diminished because of the low plasma iron concentration. The iron content of the gut remains unchanged for the first 12 hr. That the decreased absorption of iron is not caused by excessive excretion of body iron into the duodenum with dilution of the labeled test dose, is suggested by the normal quantities of iron in fecal collections. The normal iron content of intestinal mucosa with an accelerated plasma iron clearance (T/2) suggests that the decreased absorption of iron is caused by defective transfer of iron from the absorptive cell into the body. The excessive absorption of iron found in endotoxin-treated, iron-depleted rats indicates that severe iron deficiency has a greater effect upon absorption than endotoxin.

Results obtained at 24 hr show a transition between the acute and late effects of endotoxin. The serum iron concentration, plasma iron clearance (T/2), calculated iron turnover, and iron absorption studies are significantly decreased, but these changes are less marked than measurements at 12 hr. At this time the iron content of the gut becomes increased (Fig. 1). The only abnormalities observed at 48 hr are an increased intestinal iron content and decreased absorption of iron; this inverse relationship is associated with many factors which alter

iron absorption (11, 33, 34). Iron-deficient rats have decreased absorption of iron during this later period, indicating that the deposition of iron in intestinal cells may act as a regulator of absorption at that time.

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